CALIFORNIA CITRUS NURSERY BOARD

Progress Report for 2011
California Citrus Nursery Board
Lee-09; Agreement # 58-5310-9-244

Project Year 2011 Anticipated Duration of Project 3 years

Progress Report for year 3 of a 3 year project (Final report for CY2011)

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Project Title Development of reliable detection methods for Phytoplasmas from citrus and insect vectors for use in California nurseries

Objectives:

While we are preparing to combat the arrival of the psyllid in California, it is concerning to note that HLB symptoms in Brazil are associated with Phytoplasmas as well. In order to succeed in combating against emerging new disease, we believe that our efforts need to be directed at the detection of all associated organisms: Liberibacters associated with HLB, Phytoplasmas and stubborn. We are proposing to develop reliable diagnostic methods of Phytoplasma which may also be present in California and possible even associated with symptoms similar to those reported for HLB and to develop multiplex real time assays to permit concurrent detection of HLB and stubborn.

Specific objectives:

1. To conduct molecular characterization and determine the classification groups for Phytoplasma from California and isolates from Oman and Jamaica established in the *in planta* collection at the USDA ARS Exotic Disease of Citrus Quarantine facility, Beltsville, MD.

2. To develop real time PCR assays for detection of all Phytoplasma reported in citrus and additionally for specific Phytoplasmas identified from California. Develop real time assays so that they may be performed as a multiplex assay with assays for HLB, and stubborn, *Spiroplasma citri*.

3. Conduct a survey in Riverside and San Diego areas and in Lindcove area to determine if Phytoplasma occurs and if so, the incidence. Assays for HLB and *S citri* will be performed concurrently.

Progress:

Objective 1) Molecular characterization and classification of Phytoplasma
And
Objective 2) To develop real time PCR assays for detection of all Phytoplasma reported in citrus and additionally for specific Phytoplasmas identified from California.

We have established four California phytoplasma cultures *in planta* and have PCR amplified the 16S rDNA region and sequenced the amplified product to confirm the presence of phytoplasma. Additionally from the exotic citrus disease quarantine greenhouse in Beltsville, MD, we have PCR amplified and sequenced the 16S rDNA region of the witches' broom disease phytoplasma originating from Oman and a witches' broom on sweet orange originating from India. We found one apparent phytoplasma from sweet orange collected from India is, in fact, a genetic abnormality which may be graft perpetuated but not graft transmitted (e.g. the symptoms do not pass from the bud chip into the inoculated plant). The sequence information obtained has been used to develop conventional PCR assays and real time qPCR assays for phytoplasmas in a general sense and for specific phytoplasma. A more sensitive Taqman qPCR assay has been developed for stubborn as part of this current project. Application of the qPCR assays developed have enable detection of two different phytoplasmas from DNA extractions of samples collected from citrus and citrus relatives in South Florida in a survey funded by the Citrus Research Board to look specifically for HLB. The sequence information is currently being used to develop either a multiplex real time qPCR assay or to create a macro array qPCR system where the specific phytoplasmas would be assayed individually in separate wells, but in total 192 pathogens would be assayed for in duplicate (total of 384 wells). The Repository was able to purchase a 364 well qPCR machine last October, and this has afforded development of more sensitive assays using less reagents, and offers the possibility that Taqman based assays could be pre-packaged in a macro-array format so that up to 192 pathogens could be assayed from one sample at the same time. The cost of development of the macro array will largely be funded as part of a specialty crop project to develop better detection methods for graft transmissible citrus pathogens in collaboration with Drs. Yokomi and Vidaliakis.

Objective 3) conduct surveys in Riverside, San Diego, and Lindcove areas utilizing the multiplex assays for phytoplasmas, stubborn, and HLB.

We have about 6,500 DNA extractions from Southern California which have been assayed for phytoplasma, *Candidatus* Liberibacter species, and stubborn. We are holding aliquots of these extractions for use with either the multiplex qPCR assay or the macro qPCR array, we estimate testing to occur within 6 months. With the Asian citrus psyllid coming into the Riverside area, we are continuing to collect samples for testing for *Cd* L species, phytoplasma, and stubborn.